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31

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(21) International Application Number: PCT/FI95/00555 (22) International Filing Date: 9 October 1995 (09.10.95) (30) Priority Data: 08/320,432 7 October 1994 (07.10.94) US (71) Applicant: HELSINKI UNIVERSITY LICENSING LTD. OY [FI/FI]; Teollisuuskatu 23, FIN-00510 Helsinki (FI). (72) Inventor: ALITALO, Kari; Nyrikintie 4A, FIN-02100 Espoo (FI). (74) Agent: OY JALO ANT-WUORINEN AB; Iso Roobertinkatu 4-6A, FIN-00120 Helsinki (FI).			(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: CYTOPLASMIC TYROSINE KINASE (57) Abstract  Provided are cytoplasmic tyrosine kinase molecules, DNA encoding them; and methods for their use and production.			

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- 1 -

**CYTOPLASMIC TYROSINE KINASE****FIELD OF THE INVENTION**

The present invention generally relates to a novel cytoplasmic tyrosine kinase, the gene encoding it, and its use in diagnostic and therapeutic procedures.

**BACKGROUND OF THE INVENTION**

Cellular processes involved in the maintenance, differentiation, and repair of cells and tissues are regulated, in part, by intercellular and intracellular signals which are controlled by the binding of growth factors and other ligands to their receptors. One important mode of signalling used by cells to regulate gene expression and activation or deactivation of biochemical pathways is tyrosine phosphorylation. Numerous tyrosine kinases are known and they usually exist as transmembrane receptors for polypeptide growth factors, such as epidermal growth factor, insulin, insulin-like growth factor I, platelet-derived growth factors, and fibroblast growth factors. See, generally, Ullrich, et al., *Cell*, 61:243-254 (1990); and Heldin, et al., *Cell Regulation*, 1:555-556 (1990). Of interest to the present invention are several receptor tyrosine kinases which recognize hematopoietic growth factors as their ligands. These include the c-fms receptor tyrosine kinase which is the receptor for colony-stimulating factor 1, Sherr, et al., *Cell* 41: 665-676 (1985), and c-kit, a primitive and less well-characterized hematopoietic growth factor reported in Huang, et al., *Cell* 63: 225-233 (1990).

- 2 -

Tyrosine kinases are generally divided into families, subfamilies, and classes based upon structural characteristics. In general, two broad classifications exist and those include

5 membrane-bound tyrosine receptor kinases and nonreceptor tyrosine kinases, which include cytoplasmic and nuclear tyrosine kinases. An example of tyrosine receptor kinases is the family of epidermal growth factor receptor kinases which

10 form a subfamily of transmembrane receptor kinases with extracellular domains. That subfamily, along with others, such as insulin receptor kinases, contain homologous cysteine-rich repeats in their extracellular domains. See Hirai, et al., *Science*,

15 238: 1717-1720 (1987). Other membrane-bound receptor tyrosine kinases contain extracellular folds which are characteristic of the immunoglobulin superfamily.

The nonreceptor tyrosine kinases are often

20 referred to as a single group comprising Src-like kinases, but it is now recognized that the nonreceptor tyrosine kinases may be divided into several families. Bolen, *Oncogene*, 8: 2025-2031 (1993); Wang, *TIBS* 19, 373-376, 1994.

25 For example, a newly-identified non-receptor tyrosine kinase family includes three independently-cloned genes, *TEC*, *ITK* (also known as *TSK* or *EMT*), and *BTK* (formerly known as *ATK*, or *EMB*). Proteins encoded by those genes are generally

30 homologous to the *Drosophila melanogaster* Src28C tyrosine kinase. Such peptides generally contain SH3 and SH2 domains upstream of the tyrosine kinase domain. However, peptides encoded by *TEC/ITK/BTK* also typically contain a long N-terminal region

35 which does not have a consensus myristylation

- 3 -

residue which is generally conserved in Src-like kinases. Instead, the N-terminal regions of proteins of the *TEC/ITK/BTK* family contain a pleckstrin homology (PH) domain which is described in Musacchio, et al., *TIBS*, 18: 343-348 (1993). Finally, the *TEC/ITK/BTK* family generally consists of peptides which have a short C-terminus, lacking the regulatory tyrosine phosphorylation site found in most Src-like kinases.

10           The core of the pleckstrin domain is an antiparallel beta-sheet consisting of seven strands. The C-terminus is folded into a long alpha-helix. The domain is electrostatically polarized and contains a pocket which may be involved in binding to a ligand, such as a peptide or a small protein. This core structure is conserved in all PH domains, which, however, have large variations in the loops surrounding the putative binding pocket. The functions of pleckstrin domains are still unknown, although they have been shown to bind to the  $\beta$  and  $\gamma$  subunits of complex G-proteins.

20           The gene encoding the *TEC* tyrosine kinase was identified in murine hepatocarcinoma cells and was later found to be expressed in all murine hematopoietic cell lines examined. Mano, et al., *Oncogene*, 8: 417-424 (1993). The other two members of the family, *ITK* and *BTK*, are selectively expressed at certain stages of T-cell and B-cell development, respectively. Expression of the *ITK* mRNA is induced upon T-cell activation by IL-2 and mutations in *BTK* are thought to be responsible for X-linked agammaglobulinemia (Burton's disease, XLA), a disease characterized by a lack of circulating mature B-cells in affected males. Several different BTK mutations have been described in murine models

- 4 -

of XLA, including point mutations in the PH or SH2 domains, which may be involved in functionally important interactions with other proteins.

Cytoplasmic tyrosine kinases have been  
5 reported to associate with ligand-activated  
transmembrane receptors and they appear to initiate  
or to amplify ligand-induced signals. For example,  
the T- and B-cell receptors and cytokine receptors  
in hematopoietic cells associate with certain  
10 members of the Src tyrosine kinase family in order  
to transduce their signals. Upon ligand binding to  
these receptors, a rapid increase in tyrosine  
phosphorylation of specific intracellular substrates  
is observed. These signalling pathways appear  
15 therefore to depend on specific intracellular  
tyrosine kinases recruited and activated by the  
stimulated receptors. Members of the Src family are  
expressed in a cell lineage-selective manner, which  
is consistent with this hypothesis. Several  
20 cytokine receptors also interact with and activate  
JAK family of kinases, which in turn directly  
phosphorylate transcriptional regulators.

In contrast to cytokine receptors, most  
growth factor receptors contain intrinsic tyrosine  
25 kinase activity. Autophosphorylated tyrosyl  
residues of some of these receptors, such as  
platelet-derived growth factor receptor and  
hepatocyte growth factor/scatter factor receptor  
bind to SH2 domains of cytoplasmic tyrosine kinases,  
30 such as c-Src. The recruitment of a cytoplasmic  
tyrosine kinase to an activated receptor complex can  
amplify the signal by associating with other SH2  
domain-containing signal transducers and possibly  
with proteins binding to the SH3 domain.

- 5 -

The present invention provides a new cytoplasmic receptor tyrosine kinase which shares characteristics with members of the *TEC/ITK/BTK* subfamily and is useful as a marker for cell growth and differentiation and for various types of tumor formation and in the diagnostics and treatment of diseases resulting from deregulated tyrosine phosphorylation. Using a presently-claimed cytoplasmic tyrosine kinase, it is possible to isolate the growth factor or cytokine receptor whose signals are mediated through such kinases by methods standard in the art.

#### SUMMARY OF THE INVENTION

The present invention provides novel cytoplasmic tyrosine kinases capable of stimulating growth and/or proliferation of hematopoietic cells. In a preferred embodiment, a protein according to the invention is a *BMX* protein comprising the amino acid sequence shown in SEQ ID NO: 3. Also in a preferred embodiment, the invention provides cDNAs encoding *BMX*.

In a preferred embodiment, a *BMX* tyrosine kinase according to the invention comprises a fragment of the *BMX* protein which is capable of stimulating the growth and/or differentiation of hematopoietic cells, whether *in vivo* or *in vitro*. Also in a preferred embodiment, the invention provides a DNA encoding a fragment of the *BMX* protein, which fragment is capable of stimulating the growth and/or differentiation of hematopoietic cells.

The present invention also provides an antibody directed against proteins of the invention;

- 6 -

including a monoclonal antibody and a hybridoma producing it.

Methods according to the invention comprise means for detecting the growth and/or differentiation of hematopoietic cells comprising the steps of exposing tissue to a detectably-labelled DNA or antibody according to the invention; washing the tissue, and detecting any label which remains in the tissue after washing.

10 Methods for labelling DNA and protein and methods for preparing tissue for hybridization and immunohistochemistry are well-known in the art. The present invention also provides a unique tyrosine kinase, the activity of which is inhibited by

15 specific inhibitors with consequent effects on the growth or differentiation of cells expressing *BMX*.

Additional embodiments and features of the invention will become apparent to the ordinarily-skilled artisan upon consideration of the following detailed description thereof.

20

#### DESCRIPTION OF THE FIGURES

Figure 1 shows amino acid sequences of *BMX* (SEQ ID NO: 3), *BTK* (SEQ ID NO: 4), *ITK* (SEQ ID NO: 5), *TEC* (SEQ ID NO: 6), *DSrc28C* (SEQ ID NO: 8) and the consensus sequence (SEQ ID NO: 7).

25

Figure 2A is a Northern blot and hybridization analysis of polyA+ RNA from human umbilical vein endothelial cells (HUVEC) and HT-1080 human fibrosarcoma cells.

30 Figure 2B is a Western blot of anti-*BMX* and control anti-*SEX* immunoprecipitates from HUVEC cells.

Figure 2C shows anti-PTyr Western analysis of *BMX* immunoprecipitates from COS cells transfected



- 7 -

with a *BMX*-containing vector or an empty vector (MOCK).

Figure 2D shows SDS-PAGE analysis of immunoprecipitates from COS-transfected cells (*BMX*), subjected to in vitro kinase reaction.

Figure 3 is a Western blot of *BMX* retrovirus-expressing *BMX* or control virus-infected (c) NIH3T3 cells lysed directly (lanes marked "-") or immunoprecipitated (IP) using anti-*BMX* antiserum.

Figure 4 shows a normal metaphase human chromosomes and an enlargement of the X chromosome showing localization of the *BMX*-encoding gene, along with a schematic depiction of the chromosome.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention provides novel tyrosine kinases, such as the *BMX* tyrosine kinase shown in SEQ ID NO: 3; fragments of said kinases; and DNA encoding said kinases and said fragments. Such cDNAs were isolated from genomic libraries constructed from bone marrow and endothelial cell sources. The *BMX*-encoding cDNA comprises an open reading frame of 2025 bp, encoding 675 amino acids. The protein product comprises a single tyrosine kinase domain, an SH2 domain, and an SH3 domain. The tyrosine kinase domain is approximately 70% homologous to the tyrosine kinase domains of BTK, ITK, and TEC as shown by a comparison of SEQ ID NO: 3 with SEQ ID NO: 4, 5, and 6 respectively. A fragment according to the present invention is any portion of the primary structure of the intact kinase which retains the ability to stimulate or inhibit the growth and/or differentiation of hematopoietic cells.

- 8 -

A *BMX* cytoplasmic tyrosine kinase according to the invention has a deduced amino acid sequence, shown in SEQ ID NO: 3, which is closely homologous to the sequences of *Btk*, *Itk*, *Tec*, and  
5 *Drosophila melanogaster* *Src28C* tyrosine kinases. The alignment of the *BMX* sequence with its closest homologous sequences is shown in Figure 1. Inspection of that figure, suggests that the ATG codon at position 36 of the *BMX* cDNA is the  
10 translation initiation site. That codon is embedded in a kozak consensus translation initiation sequence (AATATGG).

It has been reported that the variable N-terminal domains of members of the *Src* family of  
15 kinases interact with transmembrane receptors. *Mustelin*, et al., *TIBS*: 18: 215-220 (1993). As shown in Figure 1, the N-terminal region of *BMX* has significant homology with those of *TEC*, *ITK*, and *BTK*. The N-terminal domain of *BMX* also has a region  
20 which compares to the PH (Pleckstrin Homology) consensus sequence found in various GPTase activating (GAP) proteins and in other kinases and cytoskeletal proteins. The core of the pleckstrin domain is an antiparallel beta-sheet consisting of  
25 seven strands and the C-terminal portion is folded into a long alpha helix. The Pleckstrin domain is electrostatically polarized and contains a putative ligand-binding domain.

In contrast to cytokine receptors, most  
30 growth factor receptors contain intrinsic tyrosine kinase activity. Autophosphorylated tyrosyl residues of some growth factor receptors (e.g., platelet-derived growth factor receptor) bind to SH2 domains of cytoplasmic tyrosine kinases, such as  
35 c-*Src*. The recruitment of a cytoplasmic tyrosine

- 9 -

kinases to an activated growth factor receptor complex amplifies the signal through that complex by association of the tyrosine kinase with other SH3 and SH2 containing signal transducers.

5           Claimed *BMX* proteins possess both SH2 and SH3 domains. However, the *BMX* SH3 domain varies from the consensus sequence in that it includes two strongly hydrophilic portions rich in Ser and Glu residues in its C-terminus. This distinction may be  
10           due to a splice variation. The foregoing provides substantial evidence that the inventive tyrosine kinases bind active portions of growth factor receptors and, due to the localization of claimed proteins, do so in hematopoietic cell lines.

15           Accordingly, inventive proteins are useful for stimulating hematopoietic cell lines. Moreover, inventive DNAs are useful as diagnostic reagents in the detection of hematopoietic cell proliferation and oncogenesis. Antibodies and  
20           peptides of the invention are additionally useful for inhibiting activity of growth factors. The following examples provide details of the isolation, characterization, localization, and use of inventive proteins, DNAs, and methods for use thereof.

25

**EXAMPLE 1****Cloning and Analysis of cDNA Encoding  
Proteins of the Invention**

          Total RNA was prepared from normal human bone marrow by guanidium thiocyanate extraction. An  
30           aliquot of 2  $\mu$ g RNA was then reversed-transcribed using 10U of avian myeloblastosis virus reverse transcriptase in the presence of 0.5  $\mu$ g oligo-dT primer, 1  $\mu$ M each of deoxyadenosine triphosphate, deoxyguanine triphosphate, deoxycytosine

- 10 -

triphosphate, and deoxythymidine triphosphate, and 10 U RNasin (Promega, Madison, WI). The reaction buffer contained 50 mM Tris-HCl, pH8.1, 6mM MgCl<sub>2</sub>, 40 mM KCl and 1mM dithiothreitol. The reaction contents were incubated at 42°C for 1 hour, then at 52°C for 30 minutes, and then at 95°C for 5 minutes.

Approximately 3% of the reverse transcribed cDNA was then amplified by PCR in a reaction volume of 100 µl using 3 U Dynazyme (Finnzymes, Helsinki, FI) in a reaction buffer comprising 1.5 mM MgCl<sub>2</sub> and in the presence of 200 µM each of deoxyadenosine triphosphate, deoxycytosine triphosphate, deoxyguanine triphosphate, and deoxythymidine triphosphate. The primers were 5'-GGTCTAGAA(A/g)AA(A/G)TT(C/T)GT(C/G)CAC(A/C)G(G/A)GAC-3' (0.1 µM) (SEQ ID NO: 1) and 5'-GCTCTAGA(G/A)GGCCATCCA(T/C)TT(G/C/A)AC(T/C/A)GG-3' (0.15µM) (SEQ ID NO: 2) which represented the sense and antisense primers, respectively. Both primers were obtained from conserved tyrosine kinase domains from known kinases. The protocol for amplification was 90 seconds at 95°C; 120 seconds at 42°C; 180 seconds at 68°C for 35 cycles in a volume of 100 µl in a Perkin Elmer DNA Thermo Cycler 480. A 150 bp cDNA product representing the novel *BMX*-encoding sequences was obtained. That product was subcloned into a PCR vector using a TA-cloning kit (Invitrogen) according to the Manufacturer's instruction.

The PCR-amplified *BMX* product designated B1, described above, was radiolabelled with 32pCTP using random priming and used to screen an oligo-dT-primed λgt10 cDNA library constructed from human bone marrow RNA (Clontech). The B1 cDNA

- 11 -

included the sequence obtained by PCR amplification and flanking open reading frame, predicting a cytoplasmic tyrosine kinase very closely related to the newly identified Btk kinase. When the B1 cDNA  
5 was used to probe Northern blots, no specific signal was detected in any cell lines examined. Nevertheless, according to results obtained by reverse transcription-PCR amplification of RNA, *BMX* appeared to be expressed, not only in bone marrow,  
10 but also in endothelial cells. Therefore, a human cDNA library derived from endothelial cell RNA was screened in order to obtain full length cDNA. Several positive plaques were chosen and the longest *BMX* cDNA insert of approximately 2.4 kb (E7) was  
15 subcloned into a pGEM plasmid (Promega) and sequenced on both strands using the dideoxy chain termination method of Sanger. The E7 clone was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 on October  
20 5, 1994 as accession No. ATCC 75907. Computer analyses of the sequences were performed using the GCG program as reported in Devereux, et al., *Nucl. Acids Res.*, 12: 387-395 (1984), incorporated by reference herein. The *BMX* sequence obtained is  
25 shown in Figure 1 and in SEQ ID NO: 3.

The E7 subclone contained an open reading frame capable of encoding 675 amino acids. The deduced amino acid sequence was closely homologous to the sequences of TEC, ITK, and BTK and to the  
30 *Drosophila melanogaster* Src28C tyrosine kinase. Sequence alignment suggested that the ATG at position 36 of the cDNA was the translation initiation site.

The *BMX* protein has an N-terminal region  
35 of 210 residues comprising a PH domain (shaded

- 12 -

region in Fig. 1), but no consensus myristylation site. The variable amino terminal domains of the Src family are involved in interaction with transmembrane receptors. Accordingly, it is  
5 interesting to note that the long N-terminal region of *BMX* shares a high degree of homology with the *Btk*, *Itk* and *Tec* kinases. This part of the molecule may have a role in associating with as yet unknown receptors or signal transducers. In this region, the  
10 sequences of these four tyrosine kinases are rich in basic amino acid residues and they fit the consensus for the PH domain found in a number of proteins, such as certain GTPase activating proteins (GAP) and GDP-GTP exchange factors (such as SOS1), in kinases  
15 such as *sARK* and *RAC*, and in dynamin, kinesin and spectrin.

The PH domain is followed by an SH3 and an SH2 domain (boxed region in Figure 1). For comparison, the corresponding regions of *Drosophila melanogaster* Src28C TK sequence are also shown in  
20 Figure 1. The sequences between amino acid residues 185-206 and 207-228 (horizontal arrows in Figure 1) of *BMX* are about 80% identical with each other both at the nucleotide and at the amino acid level,  
25 suggesting that this stretch originated from a duplication of the *BMX* DNA sequence. The latter stretch (207-228) belongs to the N-terminal portion of the *BMX* SH3 domain.

Although features of SH3 domains are  
30 common to all members of the *BMX/BTK/ITK/TEC* tyrosine kinase family, some of the sequences diverge from the consensus. The C-terminal portion of the *BMX* SH3 sequence (downstream of the WW motif at position 243) diverges from those of the other  
35 kinases, and contains two strongly hydrophilic

- 13 -

stretches, rich in Ser and Glu residues. In contrast, the SH2 domain is well-conserved in the *BMX* sequence when compared with the other tyrosine kinases (Fig. 1). Within the tyrosine kinase domain (arrows) the ATP binding motif containing the Gly(434)XGlyXXGly sequence and the Lys435 residue are marked in Fig 1. The Tyr566 residue of *BMX* corresponds to the conserved Tyr416 autophosphorylation site of c-Src. The catalytic domain is followed by a short C-terminal tail, where nonconserved autophosphorylation sites are found. Multiple stop codons were found after codon 675 of the *BMX* sequence.

Polyadenylated RNAs from several human fetal and adult tissues were analyzed for *BMX* RNA by Northern blotting and hybridization. *BMX* transcripts were prominent in both fetal and adult heart. Weaker signals were obtained from fetal lung and kidney; and from adult skeletal muscle, placenta, lung, liver, testis, ovary, and small and large intestine. Adult kidney, pancreas and prostate gave signals only after a very long exposure of the autoradiogram, whereas no signal could be obtained from fetal or adult brain or fetal liver or kidney. Thus, *BMX* appears to be more widely expressed than the related Btk, Itk and Tec tyrosine kinases. At least part of these hybridization signals may be derived from hematopoietic cells in these organs.

- 14 -

**EXAMPLE 2****Expression and Analysis of the BMX Protein**

A BMX retrovirus was constructed into pBABEpuro vector. The pBABEpuro vector is reported in Morgenstern, et al., *Nucl. Acids Res.*, 12: 387-395 (1990). The resulting vector was then transfected into BOSC23 cells which as reported in Pear, et al., *Proc. Natl. Acad. Sci. (USA)*, 90: 8392-8396 (1993), incorporated by reference herein.

The supernatant was then collected 48 hours later and used to infect NIH3T3 cells. The infected cells were selected by growth for 2 weeks in puromycin. COS cells were transfected with 10 µg of the pMT2 vector, which is reported in Kaufman, et al, *Cell. Biol.*, 9: 946-958, incorporated by reference herein, containing the BMX cDNA insert using the calcium phosphate method. After 36-48 hours of growth, the cells were extracted with the electrophoresis sample buffer 2.5 % sodium dodecyl sulfate, 0.125 M Tris-HCl, pH 6.8 for Western blotting or with ice-cold RIPA buffer (50mM TrisHCl pH 7.5, NaCl 150 mM, 1% Triton X100, 1% sodium deoxycholate, 0.1% SDS, containing 10 mg/ml pepstatin, 100 µg/ml leupeptin, 0.05 TIU/ml aprotinin, 1 mM PMSF and 1 mM activated sodium orthovanadate) for immunoprecipitation. The clarified supernatants were immunoprecipitated with 5 µl of anti-BMX antiserum raised in rabbits using a GST-fusion protein (Pharmacia) engineered to express BMX amino acid residues 599-675. Preimmune serum and anti-SEX antiserum against an unrelated protein (L.T., in preparation) were used as controls.

Samples were analyzed in 7.5% SDS-PAGE followed by Western blotting and detection using a



- 15 -

1: 1000 dilution of the anti-BMX antiserum (Figure 2B) or the PY20 anti-phosphotyrosine monoclonal antibodies as shown in Figure 2C (Zymed), followed by peroxidase-conjugated antibodies against mouse  
5 immunoglobulins and ECL detection according to the manufacturer's instructions (Amersham).

Alternatively, the immunoprecipitates were subjected to a kinase reaction in 25 mM HEPES, pH 7.2, 100mM NaCl, 5 mM MgCl<sub>2</sub>, 10 mM MnCl<sub>2</sub> and 10 mCi [<sup>32</sup>P]-ATP  
10 for 10 minutes at room temperature, followed by SDS-PAGE and autoradiography.

Results are shown in Figures 2A-2D.

Figure 2A shows Northern blotting analysis of RNA from human umbilical vein endothelial cells using  
15 the BMX probe (labelled BMX NB in the Figure). A 2.7 kb mRNA band is seen. Immunoprecipitation of the BMX protein followed by immunoblotting with anti-BMX antibodies resulted in the detection of a weak band of 80 kD apparent molecular weight as  
20 shown in Figure 2B and 3. That band was not seen in control immunoprecipitates. Immunoprecipitation and immunoblotting of BMX from NIH3T3 cells expressing a BMX retrovirus and from COS cells transfected with a BMX plasmid expression vector also resulted in the  
25 detection of a 80 kD polypeptide, which was tyrosyl phosphorylated as shown in Figure 2C (α-PTyr WB). However, that polypeptide was only weakly labelled in immunocomplex kinase reactions [<sup>32</sup>P]-ATP, as shown in Figure 2D.

30

### EXAMPLE 3

#### Chromosomal Localization of the BMX Gene

A Southern blot made from 24 interspecies somatic cell hybrids was obtained from the Mutant

- 16 -

Cell Repository of the Coriell Institute (Camden, NJ).

In order to determine the chromosomal localization of the *BMX* gene, DNAs from human rodent  
5 somatic cell hybrids containing defined sets of human chromosomes were analyzed by Southern blotting and hybridization with the *BMX* probe. Among 24 DNA samples, human-specific signals were observed in two human/Chinese hamster hybrids, one containing human  
10 chromosomes 1 and X and the other only the human X chromosome. This analysis indicated that the *BMX* gene is located in chromosome X. Thus the name Bone Marrow kinase gene on the X chromosome, *BMX*, was chosen. A human placenta cosmid library in pWE15,  
15 described in Lichter, et al., *Human Genet.*, 80: 224-234 (1988), and a human X-chromosome yeast artificial chromosome (YAC) library were screened with the [<sup>32</sup>P]-labelled insert of the *BMX* cDNA. Positive clones were rescreened until pure, and  
20 verified by Southern blotting and hybridization.

Slides with human metaphase chromosomes, prepared from 5-bromo deoxyuridine-synchronized lymphocyte cultures were prehybridized and  
hybridized essentially as described in Lichter, et  
25 al., *Human Genet.*, 80: 224-234 (1988), incorporated by reference herein. Four *EcoRI* fragments (1, 1.5, 2.3 and 2.5 kb) of the *BMX* cosmid were used as probes after labeling with biotin-16-dUTP by nick translation. The four probes were pooled in a  
30 mixture of 50% formamide, 2xSSC, 1% Tween 20, 10% dextran sulfate, 25µg Cot-I DNA and 8 mg salmon sperm DNA, denatured at 75 °C for 5 minutes and then preannealed at 37 °C for 30 minutes. After hybridization, the slides were stringently washed,

- 17 -

the signal was made fluorescent, amplified, and the chromosomes were counterstained with propidiumiodide and DAPI. The results were analyzed and photographed in a confocal laser scanning microscope (Zeiss).

5 A genomic cosmid clone isolated by hybridization with the *BMX* cDNA gave signals from chromosomes X and 18 in the hybrid panel. Therefore, all four *BMX*-positive *EcoRI* fragments of  
10 this clone were labelled and used inflorescence in situ hybridization to further localize the *BMX* gene. This probe gave a specific signal in Xp22.2-p21 (Fig. 4). The *BMX* cDNA was then hybridized to YACs from the Xp21 and Xp22 region. The 400 kb ICRF YAC  
15 900G1096 and the 350 kb CEPH YAC244G7 were positive for *BMX*. Both of these YACs were non-chimeric as analyzed by FISH; the former was positive for the DXS207 and DXS197 loci and the latter for only DXS197. As *BMX* was negative on YACs positive for  
20 DXS197 and DXS43, this maps *BMX* between the DXS197 and DXS207 loci in band Xp22.2.

The invention has been described in terms of its preferred embodiments. Accordingly,  
additional aspects of the invention will be apparent  
25 to the ordinarily-skilled artisan upon reading the present application.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Helsinki University Licensing Ltd Oy
- (ii) TITLE OF INVENTION: Cytoplasmic Tyrosine Kinase
- (iii) NUMBER OF SEQUENCES: 8
- (iv) CORRESPONDENCE ADDRESS:
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  - (C) CITY: Helsinki
  - (E) COUNTRY: Finland
  - (F) ZIP: FIN-00120
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
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  - (C) TELEX: 123505 JALO FI

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 28 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGTCTAGAAR AARTTYGTSC ACMGRGAC

28

## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GCTCTAGARG GCCATCCAYT TVACHGG

27

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 675 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

Met Asp Thr Lys Ser Ile Leu Glu Glu Leu Leu Lys Arg Ser Gln
 1           5           10           15
Gln Lys Lys Lys Met Ser Pro Asn Asn Tyr Lys Glu Arg Leu Phe Val
          20           25           30
Leu Thr Lys Thr Asn Leu Ser Tyr Tyr Glu Tyr Asp Lys Met Lys Arg
      35           40           45
Gly Ser Arg Lys Gly Ser Ile Glu Ile Lys Lys Ile Arg Cys Val Glu
 50           55           60
Lys Val Asn Leu Glu Glu Gln Thr Pro Val Glu Arg Gln Tyr Pro Phe
 65           70           75           80
Gln Ile Val Tyr Lys Asp Gly Leu Leu Tyr Val Tyr Ala Ser Asn Glu
          85           90           95
Glu Ser Arg Ser Gln Trp Leu Lys Ala Leu Gln Lys Glu Ile Arg Gly
      100          105          110
Asn Pro His Leu Leu Val Lys Tyr His Ser Gly Phe Phe Val Asp Gly
      115          120          125
Lys Phe Leu Cys Cys Gln Gln Ser Cys Lys Ala Ala Pro Gly Cys Thr
      130          135          140
Leu Trp Glu Ala Tyr Ala Asn Leu His Thr Ala Val Asn Glu Glu Lys
      145          150          155          160
His Arg Val Pro Thr Phe Pro Asp Arg Val Leu Lys Ile Pro Arg Ala
      165          170          175
Val Pro Val Leu Lys Met Asp Ala Pro Ser Ser Ser Thr Thr Leu Ala
      180          185          190
Gln Tyr Asp Asn Glu Ser Lys Lys Asn Tyr Gly Ser Gln Pro Pro Ser
      195          200          205
Ser Ser Thr Ser Leu Ala Gln Tyr Asp Ser Asn Ser Lys Lys Ile Tyr
      210          215          220
Gly Ser Gln Pro Asn Phe Asn Met Gln Tyr Ile Pro Arg Glu Asp Phe
      225          230          235          240
Pro Asp Trp Trp Gln Val Arg Lys Leu Lys Ser Ser Ser Ser Ser Glu
      245          250          255

```

Asp Val Ala Ser Ser Asn Gln Lys Glu Arg Asn Val Asn His Thr Thr  
 260 265 270  
 Ser Lys Ile Ser Trp Glu Phe Pro Glu Ser Ser Ser Ser Glu Glu Glu  
 275 280 285  
 Glu Asn Leu Asp Asp Tyr Asp Trp Phe Ala Gly Asn Ile Ser Arg Ser  
 290 295 300  
 Gln Ser Glu Gln Leu Leu Arg Gln Lys Gly Lys Glu Gly Ala Phe Met  
 305 310 315 320  
 Val Arg Asn Ser Ser Gln Val Gly Met Tyr Thr Val Ser Leu Phe Ser  
 325 330 335  
 Lys Ala Val Asn Asp Lys Lys Gly Thr Val Lys His Tyr His Val His  
 340 345 350  
 Thr Asn Ala Glu Asn Lys Leu Tyr Leu Ala Glu Asn Tyr Cys Phe Asp  
 355 360 365  
 Ser Ile Pro Lys Leu Ile His Tyr His Gln His Asn Ser Ala Gly Met  
 370 375 380  
 Ile Thr Arg Leu Arg His Pro Val Ser Thr Lys Ala Asn Lys Val Pro  
 385 390 395 400  
 Asp Ser Val Ser Leu Gly Asn Gly Ile Trp Glu Leu Lys Arg Glu Glu  
 405 410 415  
 Ile Thr Leu Leu Lys Glu Leu Gly Ser Gly Gln Phe Gly Val Val Gln  
 420 425 430  
 Leu Gly Lys Trp Lys Gly Gln Tyr Asp Val Ala Val Lys Met Ile Lys  
 435 440 445  
 Glu Gly Ser Met Ser Glu Asp Glu Phe Phe Gln Glu Ala Gln Thr Met  
 450 455 460  
 Met Lys Leu Ser His Pro Lys Leu Val Lys Phe Tyr Gly Val Cys Ser  
 465 470 475 480  
 Lys Glu Tyr Pro Ile Tyr Ile Val Thr Glu Tyr Ile Ser Asn Gly Cys  
 485 490 495  
 Leu Leu Asn Tyr Leu Arg Ser His Gly Lys Gly Leu Glu Pro Ser Gln  
 500 505 510  
 Leu Leu Glu Met Cys Tyr Asp Val Cys Glu Gly Met Ala Phe Leu Glu  
 515 520 525  
 Ser His Gln Phe Ile His Arg Asp Leu Ala Ala Arg Asn Cys Leu Val  
 530 535 540  
 Asp Arg Asp Leu Cys Val Lys Val Ser Asp Phe Gly Met Thr Arg Tyr  
 545 550 555 560  
 Val Leu Asp Asp Gln Tyr Val Ser Ser Val Gly Thr Lys Phe Pro Val  
 565 570 575  
 Lys Trp Ser Ala Pro Glu Val Phe His Tyr Phe Lys Tyr Ser Ser Lys  
 580 585 590  
 Ser Asp Val Trp Ala Phe Gly Ile Leu Met Trp Glu Val Phe Ser Leu  
 595 600 605

21

Gly Lys Gln Pro Tyr Asp Leu Tyr Asp Asn Ser Gln Val Val Leu Lys  
 610 615 620

Val Ser Gln Gly His Arg Leu Tyr Arg Pro His Leu Ala Ser Asp Thr  
 625 630 635 640

Ile Tyr Gln Ile Met Tyr Ser Cys Trp His Glu Leu Pro Glu Lys Arg  
 645 650 655

Pro Thr Phe Gln Gln Leu Leu Ser Ser Ile Glu Pro Leu Arg Glu Lys  
 660 665 670

Asp Lys His  
 675

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 659 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Ala Val Ile Leu Glu Ser Ile Phe Leu Lys Arg Ser Gln Gln  
 1 5 10 15

Lys Lys Lys Thr Ser Pro Leu Asn Phe Lys Lys Arg Leu Phe Leu Leu  
 20 25 30

Thr Val His Lys Leu Ser Tyr Tyr Glu Tyr Asp Phe Glu Arg Gly Arg  
 35 40 45

Arg Gly Ser Lys Lys Gly Ser Ile Asp Val Glu Lys Ile Thr Cys Val  
 50 55 60

Glu Thr Val Val Pro Glu Lys Asn Pro Pro Pro Glu Arg Gln Ile Pro  
 65 70 75 80

Arg Arg Gly Glu Glu Ser Ser Glu Met Glu Gln Ile Ser Ile Ile Glu  
 85 90 95

Arg Phe Pro Tyr Pro Phe Gln Val Val Tyr Asp Glu Gly Pro Leu Tyr  
 100 105 110

Val Phe Ser Pro Thr Glu Glu Leu Arg Lys Arg Trp Ile His Gln Leu  
 115 120 125

Lys Asn Val Ile Arg Tyr Asn Ser Asp Leu Val Gln Lys Tyr His Pro  
 130 135 140

Cys Phe Trp Ile Asp Gly Gln Tyr Leu Cys Cys Ser Gln Thr Ala Lys  
 145 150 155 160

Asn Ala Met Gly Cys Gln Ile Leu Glu Asn Arg Asn Gly Ser Leu Lys  
 165 170 175

Pro Gly Ser Ser His Arg Lys Thr Lys Lys Pro Leu Pro Pro Thr Pro  
 180 185 190

Glu Glu Asp Gln Ile Leu Lys Lys Pro Leu Pro Pro Glu Pro Ala Ala  
 195 200 205  
 Ala Pro Val Ser Thr Ser Glu Leu Lys Lys Val Val Ala Leu Tyr Asp  
 210 215 220  
 Tyr Met Pro Met Asn Ala Asn Asp Leu Gln Leu Arg Lys Gly Asp Glu  
 225 230 235 240  
 Tyr Phe Ile Leu Glu Glu Ser Asn Leu Pro Trp Trp Arg Ala Arg Asp  
 245 250 255  
 Lys Asn Gly Gln Glu Gly Tyr Ile Pro Ser Asn Tyr Val Thr Glu Ala  
 260 265 270  
 Glu Asp Ser Ile Glu Met Tyr Glu Trp Tyr Ser Lys His Met Thr Arg  
 275 280 285  
 Ser Gln Ala Glu Gln Leu Leu Lys Gln Glu Gly Lys Glu Gly Gly Phe  
 290 295 300  
 Ile Val Arg Asp Ser Ser Lys Ala Gly Lys Tyr Thr Val Ser Val Phe  
 305 310 315 320  
 Ala Lys Ser Thr Gly Asp Pro Gln Gly Val Ile Arg His Tyr Val Val  
 325 330 335  
 Cys Ser Thr Pro Gln Ser Gln Tyr Tyr Leu Ala Glu Lys His Leu Phe  
 340 345 350  
 Ser Thr Ile Pro Glu Leu Ile Asn Tyr His Gln His Asn Ser Ala Gly  
 355 360 365  
 Leu Ile Ser Arg Leu Lys Tyr Pro Val Ser Gln Gln Asn Lys Asn Ala  
 370 375 380  
 Pro Ser Thr Ala Gly Leu Gly Tyr Gly Ser Trp Glu Ile Asp Pro Lys  
 385 390 395 400  
 Asp Leu Thr Phe Leu Lys Glu Leu Gly Thr Gly Gln Phe Gly Val Val  
 405 410 415  
 Lys Tyr Gly Lys Trp Arg Gly Gln Tyr Asp Val Ala Ile Lys Met Ile  
 420 425 430  
 Lys Glu Gly Ser Met Ser Glu Asp Glu Phe Ile Glu Glu Ala Lys Val  
 435 440 445  
 Met Met Asn Leu Ser His Glu Lys Leu Val Gln Leu Tyr Gly Val Cys  
 450 455 460  
 Thr Lys Gln Arg Pro Ile Phe Ile Ile Thr Glu Tyr Met Ala Asn Gly  
 465 470 475 480  
 Cys Leu Leu Asn Tyr Leu Arg Glu Met Arg His Arg Phe Gln Thr Gln  
 485 490 495  
 Gln Leu Leu Glu Met Cys Lys Asp Val Cys Glu Ala Met Glu Tyr Leu  
 500 505 510  
 Glu Ser Lys Gln Phe Leu His Arg Asp Leu Ala Ala Arg Asn Cys Leu  
 515 520 525  
 Val Asn Asp Gln Gly Val Val Lys Val Ser Asp Phe Gly Leu Ser Arg  
 530 535 540



Tyr Val Leu Asp Asp Glu Tyr Thr Ser Ser Val Gly Ser Lys Phe Pro  
 545 550 555 560  
 Val Arg Trp Ser Pro Pro Glu Val Leu Met Tyr Ser Lys Phe Ser Ser  
 565 570 575  
 Lys Ser Asp Ile Trp Ala Phe Gly Val Leu Met Trp Glu Ile Tyr Ser  
 580 585 590  
 Leu Gly Lys Met Pro Tyr Glu Arg Phe Thr Asn Ser Glu Thr Ala Glu  
 595 600 605  
 His Ile Ala Gln Gly Leu Arg Leu Tyr Arg Pro His Leu Ala Ser Glu  
 610 615 620  
 Lys Val Tyr Thr Ile Met Tyr Ser Cys Trp His Glu Lys Ala Asp Glu  
 625 630 635 640  
 Arg Pro Thr Phe Lys Ile Leu Leu Ser Asn Ile Leu Asp Val Met Asp  
 645 650 655  
 Glu Glu Ser

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 620 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Asn Asn Phe Ile Leu Leu Glu Glu Gln Leu Ile Lys Lys Ser Gln  
 1 5 10 15  
 Gln Lys Arg Arg Thr Ser Pro Ser Asn Phe Lys Val Arg Phe Phe Val  
 20 25 30  
 Leu Thr Lys Ala Ser Leu Ala Tyr Phe Glu Asp Arg His Gly Lys Lys  
 35 40 45  
 Arg Thr Leu Lys Gly Ser Ile Glu Leu Ser Arg Ile Lys Cys Val Glu  
 50 55 60  
 Ile Val Lys Ser Asp Ile Ser Ile Pro Cys His Tyr Lys Tyr Pro Phe  
 65 70 75 80  
 Gln Val Val His Asp Asn Tyr Leu Leu Tyr Val Phe Ala Pro Asp Arg  
 85 90 95  
 Glu Ser Arg Gln Arg Trp Val Leu Ala Leu Lys Glu Glu Thr Arg Asn  
 100 105 110  
 Asn Asn Ser Leu Val Pro Lys Tyr His Pro Asn Phe Trp Met Asp Gly  
 115 120 125  
 Lys Trp Arg Cys Cys Ser Gln Leu Glu Lys Leu Ala Thr Gly Cys Ala  
 130 135 140

Gln Tyr Asp Pro Thr Lys Asn Ala Ser Lys Lys Pro Leu Pro Pro Thr  
 145 150 155 160  
 Pro Glu Asp Asn Arg Arg Pro Leu Trp Glu Pro Glu Glu Thr Val Val  
 165 170 175  
 Ile Ala Leu Tyr Asp Tyr Gln Thr Asn Asp Pro Gln Glu Leu Ala Leu  
 180 185 190  
 Arg Arg Asn Glu Glu Tyr Cys Leu Leu Asp Ser Ser Glu Ile His Trp  
 195 200 205  
 Trp Arg Val Gln Asp Arg Asn Gly His Glu Gly Tyr Val Pro Ser Ser  
 210 215 220  
 Tyr Leu Val Glu Lys Ser Pro Asn Asn Leu Glu Thr Tyr Glu Trp Tyr  
 225 230 235 240  
 Asn Lys Ser Ile Ser Arg Asp Lys Ala Glu Lys Leu Leu Leu Asp Thr  
 245 250 255  
 Gly Lys Glu Gly Ala Phe Met Val Arg Asp Ser Arg Thr Ala Gly Thr  
 260 265 270  
 Tyr Thr Val Ser Val Phe Thr Lys Ala Val Val Ser Glu Asn Asn Pro  
 275 280 285  
 Cys Ile Lys His Tyr His Ile Lys Glu Thr Asn Asp Asn Pro Lys Arg  
 290 295 300  
 Tyr Tyr Val Ala Glu Lys Tyr Val Phe Asp Ser Ile Pro Leu Leu Ile  
 305 310 315 320  
 Asn Tyr His Gln His Asn Gly Gly Gly Leu Val Thr Arg Leu Arg Tyr  
 325 330 335  
 Pro Val Cys Phe Gly Arg Gln Lys Ala Pro Val Thr Ala Gly Leu Arg  
 340 345 350  
 Tyr Gly Lys Trp Val Ile Asp Pro Ser Glu Leu Thr Phe Val Gln Glu  
 355 360 365  
 Ile Gly Ser Gly Gln Phe Gly Leu Val His Leu Gly Tyr Trp Leu Asn  
 370 375 380  
 Lys Asp Lys Val Ala Ile Lys Thr Ile Arg Glu Gly Ala Met Ser Glu  
 385 390 395 400  
 Glu Asp Phe Ile Glu Glu Ala Glu Val Met Met Lys Leu Ser His Pro  
 405 410 415  
 Lys Leu Val Gln Leu Tyr Gly Val Cys Leu Glu Gln Ala Pro Ile Cys  
 420 425 430  
 Leu Val Phe Glu Phe Met Glu His Gly Cys Leu Ser Asp Tyr Leu Arg  
 435 440 445  
 Thr Gln Arg Gly Leu Phe Ala Ala Glu Thr Leu Leu Gly Met Cys Leu  
 450 455 460  
 Asp Val Cys Glu Gly Met Ala Tyr Leu Glu Glu Ala Cys Val Ile His  
 465 470 475 480  
 Arg Asp Leu Ala Ala Arg Asn Cys Leu Val Gly Glu Asn Gln Val Ile  
 485 490 495

25

Lys Val Ser Asp Phe Gly Met Thr Arg Phe Val Leu Asp Asp Gln Tyr  
 500 505 510  
 Thr Ser Ser Thr Gly Thr Lys Phe Pro Val Lys Trp Ala Ser Pro Glu  
 515 520 525  
 Val Phe Ser Phe Ser Arg Tyr Ser Ser Lys Ser Asp Val Trp Ser Phe  
 530 535 540  
 Gly Val Leu Met Trp Glu Val Phe Ser Glu Gly Lys Ile Pro Tyr Glu  
 545 550 555 560  
 Asn Arg Ser Asn Ser Glu Val Val Glu Asp Ile Ser Thr Gly Phe Arg  
 565 570 575  
 Leu Tyr Lys Pro Arg Leu Ala Ser Thr His Val Tyr Gln Ile Met Asn  
 580 585 590  
 His Cys Trp Lys Glu Arg Pro Glu Asp Arg Pro Ala Phe Ser Arg Leu  
 595 600 605  
 Leu Arg Gln Leu Ala Glu Ile Ala Glu Ser Gly Leu  
 610 615 620

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 630 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Asn Phe Asn Thr Ile Leu Glu Glu Ile Leu Ile Lys Arg Ser Gln  
 1 5 10 15  
 Gln Lys Lys Lys Thr Ser Leu Leu Asn Tyr Lys Glu Arg Leu Cys Val  
 20 25 30  
 Leu Pro Lys Ser Val Leu Ser Tyr Tyr Glu Gly Arg Ala Glu Lys Lys  
 35 40 45  
 Tyr Arg Lys Gly Val Ile Asp Ile Ser Lys Ile Lys Cys Val Glu Ile  
 50 55 60  
 Val Lys Asn Asp Asp Gly Val Ile Pro Cys Gln Asn Lys Phe Pro Phe  
 65 70 75 80  
 Gln Val Val His Asp Ala Asn Thr Leu Tyr Ile Phe Ala Pro Ser Pro  
 85 90 95  
 Gln Ser Arg Asp Arg Trp Val Lys Lys Leu Lys Glu Glu Ile Lys Asn  
 100 105 110  
 Asn Asn Asn Ile Met Ile Lys Tyr His Pro Lys Phe Trp Ala Asp Gly  
 115 120 125  
 Ser Tyr Gln Cys Cys Arg Gln Thr Glu Lys Leu Ala Pro Gly Cys Glu  
 130 135 140

Lys Tyr Asn Leu Phe Glu Ser Ser Ile Arg Lys Thr Leu Pro Pro Ala  
 145 150 155 160  
 Pro Glu Ile Lys Lys Arg Arg Pro Pro Pro Pro Ile Pro Pro Glu Lys  
 165 170 175  
 Lys Asn Thr Glu Glu Ile Val Val Ala Met Tyr Asp Phe Gln Ala Thr  
 180 185 190  
 Glu Ala His Asp Leu Arg Leu Glu Arg Gly Gln Glu Tyr Ile Ile Leu  
 195 200 205  
 Glu Lys Asn Asp Leu His Trp Trp Arg Ala Arg Asp Lys Tyr Gly Ser  
 210 215 220  
 Glu Gly Tyr Ile Pro Ser Asn Tyr Val Thr Gly Lys Lys Ser Asn Asn  
 225 230 235 240  
 Leu Asp Gln Tyr Glu Trp Tyr Cys Arg Asn Thr Asn Arg Ser Lys Ala  
 245 250 255  
 Glu Gln Leu Leu Arg Thr Glu Asp Lys Glu Gly Gly Phe Met Val Arg  
 260 265 270  
 Asp Ser Ser Gln Pro Gly Leu Tyr Thr Val Ser Leu Tyr Thr Lys Phe  
 275 280 285  
 Gly Gly Glu Gly Ser Ser Gly Phe Arg His Tyr His Ile Lys Glu Thr  
 290 295 300  
 Ala Thr Ser Pro Lys Lys Tyr Tyr Leu Ala Glu Lys His Ala Phe Gly  
 305 310 315 320  
 Ser Ile Pro Glu Ile Ile Glu Tyr His Lys His Asn Ala Ala Gly Leu  
 325 330 335  
 Val Thr Arg Leu Arg Tyr Pro Val Ser Thr Lys Gly Lys Asn Ala Pro  
 340 345 350  
 Thr Thr Ala Gly Phe Ser Tyr Asp Lys Trp Glu Ile Asn Pro Ser Glu  
 355 360 365  
 Leu Thr Phe Met Arg Glu Leu Gly Ser Gly Leu Phe Gly Val Val Arg  
 370 375 380  
 Leu Gly Lys Trp Arg Ala Gln Tyr Lys Val Ala Ile Lys Ala Ile Arg  
 385 390 395 400  
 Glu Gly Ala Met Cys Glu Glu Asp Phe Ile Glu Glu Ala Lys Val Met  
 405 410 415  
 Met Lys Leu Thr His Pro Lys Leu Val Gln Leu Tyr Gly Val Cys Thr  
 420 425 430  
 Gln Gln Lys Pro Ile Tyr Ile Val Thr Glu Phe Met Glu Arg Gly Cys  
 435 440 445  
 Leu Leu Asn Phe Leu Arg Gln Arg Gln Gly His Phe Ser Arg Asp Met  
 450 455 460  
 Leu Leu Ser Met Cys Gln Asp Val Cys Glu Gly Met Glu Tyr Leu Glu  
 465 470 475 480  
 Arg Asn Ser Phe Ile His Arg Asp Leu Ala Ala Arg Asn Cys Leu Val  
 485 490 495

27

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Asn Glu Ala Gly Val Val Lys Val Ser Asp Phe Gly Met Ala Arg Tyr
      500                      505                      510
Val Leu Asp Asp Gln Tyr Thr Ser Ser Ser Gly Ala Lys Phe Pro Val
      515                      520                      525
Lys Trp Cys Pro Pro Glu Val Phe Asn Tyr Ser Arg Gly Ser Ser Lys
      530                      535                      540
Ser Asp Val Trp Ser Phe Gly Val Leu Met Trp Glu Ile Phe Thr Glu
545                      550                      555                      560
Gly Arg Met Pro Phe Glu Lys Asn Thr Asn Tyr Glu Val Val Thr Met
      565                      570                      575
Val Thr Arg Gly His Arg Leu His Arg Pro Lys Leu Ala Thr Lys Tyr
      580                      585                      590
Leu Tyr Glu Val Met Leu Arg Cys Trp Gln Glu Arg Pro Glu Gly Arg
      595                      600                      605
Pro Ser Phe Glu Asp Leu Leu Arg Thr Ile Asp Glu Leu Val Glu Cys
      610                      615                      620
Glu Glu Thr Phe Gly Arg
625                      630

```

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 85 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

Val Ile Lys Glu Gly Leu Lys Lys Trp Lys Arg Phe Val Leu Leu Ser
1           5           10           15
Tyr Tyr Lys Gly Leu Ile Asp Leu Ile Ile Val Glu Phe Ile Val Leu
      20           25           30
Ile Leu Ala Glu Glu Glu Arg Trp Val Ala Leu Ile Ala Leu Tyr Asp
      35           40           45
Tyr Asp Leu Leu Gly Asp Ile Leu Trp Trp Gly Pro Tyr Val Trp Ile
      50           55           60
Ser Arg Ala Leu Leu Gly Phe Leu Val Arg Gly Tyr Ser Val His Tyr
      65           70           75           80
Phe Leu Ile Pro Val
      85

```

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 441 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```

Val Val Ala Leu Tyr Leu Gly Lys Ala Ile Glu Gly Gly Asp Leu Ser
 1           5           10           15
Val Gly Glu Lys Asn Ala Glu Tyr Glu Val Ile Asp Asp Ser Gln Glu
 20           25           30
His Trp Trp Lys Val Lys Asp Ala Leu Gly Asn Val Gly Tyr Ile Pro
 35           40           45
Ser Asn Tyr Val Gln Ala Glu Ala Leu Leu Gly Leu Glu Arg Tyr Glu
 50           55           60
Trp Tyr Val Gly Tyr Met Ser Arg Gln Arg Ala Glu Ser Leu Leu Lys
 65           70           75           80
Gln Gly Asp Lys Glu Gly Cys Phe Val Val Arg Lys Ser Ser Thr Lys
 85           90           95
Gly Leu Tyr Thr Leu Ser Leu His Thr Lys Val Pro Gln Ser His Val
 100          105          110
Lys His Tyr His Ile Lys Gln Asn Ala Arg Cys Glu Tyr Tyr Leu Ser
 115          120          125
Glu Lys His Cys Cys Glu Thr Ile Pro Asp Leu Ile Asn Tyr His Arg
 130          135          140
His Asn Ser Ala Gly Leu Ala Cys Arg Leu Lys Ser Ser Pro Cys Asp
 145          150          155          160
Arg Pro Val Pro Pro Thr Ala Gly Leu Ser His Asp Lys Trp Glu Ile
 165          170          175
His Pro Ile Gln Leu Met Leu Met Glu Glu Leu Gly Ser Gly Gln Phe
 180          185          190
Gly Val Val Arg Arg Gly Lys Trp Arg Gly Ser Ile Asp Thr Ala Val
 195          200          205
Lys Met Met Lys Glu Gly Thr Met Ser Glu Asp Asp Phe Ile Glu Glu
 210          215          220
Ala Lys Val Met Thr Lys Leu Gln His Pro Asn Leu Val Gln Leu Tyr
 225          230          235          240
Gly Val Cys Thr Lys His Arg Pro Ile Tyr Ile Val Thr Glu Tyr Met
 245          250          255
Lys His Gly Ser Leu Leu Asn Tyr Leu Arg Arg His Glu Lys Thr Leu
 260          265          270
Ile Gly Asn Met Gly Leu Leu Leu Asp Met Cys Ile Gln Val Ser Lys
 275          280          285
Gly Met Thr Tyr Leu Glu Arg His Asn Tyr Ile His Arg Asp Leu Ala
 290          295          300
Ala Arg Asn Cys Leu Val Gly Ser Glu Asn Val Val Lys Val Ala Asp
 305          310          315          320

```

Phe	Gly	Leu	Ala	Arg	Tyr	Val	Leu	Asp	Asp	Gln	Tyr	Thr	Ser	Ser	Gly	325	330	335
Gly	Thr	Lys	Phe	Pro	Ile	Lys	Trp	Ala	Pro	Pro	Glu	Val	Leu	Asn	Tyr	340	345	350
Thr	Arg	Phe	Ser	Ser	Lys	Ser	Asp	Val	Trp	Ala	Tyr	Gly	Val	Leu	Met	355	360	365
Trp	Glu	Ile	Phe	Thr	Cys	Gly	Lys	Met	Pro	Tyr	Gly	Arg	Leu	Lys	Asn	370	375	380
Thr	Glu	Val	Val	Glu	Arg	Val	Gln	Arg	Gly	Ile	Ile	Leu	Glu	Lys	Pro	385	390	400
Lys	Ser	Cys	Ala	Lys	Glu	Ile	Tyr	Asp	Val	Met	Lys	Leu	Cys	Trp	Ser	405	410	415
His	Gly	Pro	Glu	Glu	Arg	Pro	Ala	Phe	Arg	Val	Leu	Met	Asp	Gln	Leu	420	425	430
Ala	Leu	Val	Ala	Gln	Thr	Leu	Thr	Asp								435	440	

- 30 -

**CLAIMS**

What is claimed is:

1. A protein comprising the amino acid sequence shown in SEQ ID NO: 3.
2. A fragment of the protein according to claim 1 which is capable of stimulating growth of hematopoietic cells.
3. A DNA encoding the protein according to claim 1.
4. A DNA encoding the protein fragment according to claim 2.
5. A method for detecting growth of hematopoietic cells, comprising the steps of
  - (a) exposing tissue comprising said hematopoietic cells to a detectably labelled DNA according to claim 3;
  - (b) washing said tissue; and
  - (c) detecting said label in said tissue.
6. An antibody which is specifically reactive with the protein according to claim 1.



1/7

....Bmx	MDTKSILEEL	LLKRSQQKKK	MSPNNYKERL	FVLTKTNLSY	YEYD--KM
....Btk	M.AAVILESI	FLKRSQQKKK	TSPLNFKKRL	FLLTVHKLSY	YEYDFERG
....Emt	MNFIILLEEQ	LIKRSQQKRR	TSPSNFKVRF	FVLTKASLAY	FED--RHG
....Tec	MNFNTILEEI	LIKRSQQKKK	TSLNLYKERL	CVLPKSVLSY	YEG---RA
consens	....vikeg.	l.kk.....	....wk.r.	fv1....lsy	Y.....
....Bmx	-----	YPFQIVYKDG	LLVYASNEE	SRSQWLKALQ	KEIRGNPH
....Btk	EQISIIERFP	YPFQVVYDEG	PLYVFSPTTE	LKRKWIHQK	NVIRYNSD
....Emt	SDISIPCHYK	YPFQVVHDNY	LLYVFAPDRE	SRQRWVLALK	EETRNNNS
....Tec	DDGVIPCNK	FPFQVVHDAN	TLYIFAPSPQ	SRDRWVKKLL	EEIKNNNN
consens	.....	f.iv....	.lil.a...ee	er..wv.al.	..i.....
....Bmx	EKHRVPTFP	DRVLIKIPRAV	PVLKMDAPSS	STTLAQYDNE	SKKNYGSQ
....Btk	SHRKTKKPLP	PTPEEDQILK	KPLPPEPAAA	PVSTSELKK-	-----
....Emt	----SKKPLP	PTPED---NR	R-----PLWE	PEET-----	-----
....Tec	----IRKTLP	PAPEI---K	KRRPPPPIPP	EKKNTTEEI-	-----
DSrc28C	(./.)----	-----	-----	-----	-----
consens	.....	.....	.....	.....	.....
....Bmx	VRKLKSSSSS	EDVASSNQKE	RNVNHTTSKI	SWEFPESSSS	EEENLDD
....Btk	ARDKNGQEGY	IPSNVYTEA.	-----	-----	--EDSIEM
....Emt	VQDRNGHEGY	VPSSYLVEKS	-----	-----	--PNNLET
....Tec	ARDKYGSEGY	IPSNVVTGK-	-----	-----	-KSNLDDQ
DSrc28C	VKDALGNVGY	IPSNVVOAE-	-----	-----	-ALLGLER
consens	.....g.	.p...yv....	.....	.....	.....
....Bmx	SKA-VNDKKG	TVKHYHVHTN	AEN--KLYLA	ENYCFDSIPK	LIHYHQHN

Figure 1  
(1/4)

SUBSTITUTE SHEET

# PH

KR	GSRKGSIEIK	KIRCVEKVN	EEQTPVERQ-	77
RR	GSKKGSIDVE	KITCNETVVP	EKNPPPERQI	89
KK	RTLKGSIELS	RIKCVIEVK-	-----	67
EK	KYRKGVIDIS	KIKCVIEVKN	-----	67
..	...kglidl.	.i.ive....	.....	..
LL	VKYHSGFFVD	GKFLCCQCS	KAAPGCTLWE	157
LV	QKYHPCFWID	GQYLCCSQTA	KNAMGCQILE	179
LV	PKYHPNFWMD	GKWRCCSQLE	KLATGCAQYD	152
IM	IKYHPKFWAD	GSYQCCRQTE	KLAPGCEKYN	157
..	.....	.....	.....	..

# SH3

PP	SSSTSLAQYD	SNSKKIYGSQ	-PNFNMQYIP	RED-FPDWWQ	245
--	---VVALYD	YMPMNANDLQ	-LRKGDEYFI	LEESNLPWWR	253
--	---VVALYD	YQTNDPQELA	-LRNREEYCL	LDSEIHWWR	210
--	---VVALYD	FQATEAHLR	-LERGQEIYII	LEKNDLHWWR	217
--	---VVALYD	GKAIEGGDLS	VGEKNAEYEV	IDDSQEHWWK	185
..	.....alyd	y.....dl.	.l..gd...i	l.....ww.	..

# SH2

YD	WFAGNISRSQ	SEQLLRQKQK	EGAFMVRNSS	QVGMVTVSLF	335
YE	WYSKHMTRSQ	AEQLLKQEGK	EGGFIVRDSS	KAGKYTVSVF	320
YE	WYNKSISRDK	AEKLLLDTGK	EGAFMVRDSR	TAGTYTVSVF	278
YE	WYCRNTNRSK	AEQLLRTEDEK	EGGFIVRDSS	QPGLYTVSLY	275
YE	WYVGYMSRQR	AESLLKQGDK	EGCFVVRKSS	TKGLYTLSLH	253
..	w.....isr..	a...ll.....	.g.flvr...	..g.y...s...	..

Figure 1  
(2/4)

SA	GMITRLRHPV	STK-ANKVPD	SVSLNGNGIWE	LKREEITLLK	421
----	------------	------------	-------------	------------	-----

.... Btk	AKS-TGDPQG	VIRHYVVCST	PQS--QYLA	EKHLFSTIPE	LINYHQHN
.... Emt	TKAVVSENNP	CIKHYHIKET	NDNPKRYVVA	EKYVFD SIPL	LINYHQHN
.... Tec	TKF-GGEGSS	GFRHYHIKET	ATSPKYYLA	EKHAFGSIPE	IIEYHKHN
DSrc28C	TKV-----PQS	HVKHYHIKON	A--RCEYLS	EKHCCETIPD	LINYHRHN
consens	.....	.v.hy.....	.....	.....f.....	li.....

ATP binding

.... Bmx	ELGSGQFGVV	QLGKWKQGYD	VAVKMIKEGS	MSEDEFFQEA	QTMMKLSH
.... Btk	ELGTGQFGVV	KYGKWRGQYD	VAIKMIKEGS	MSEDEFIEEA	KVMNLSH
.... Emt	EIGSGQFGLV	HLGYWLNKDK	VAIKTIREGA	MSEEDFIEEA	EVMMLSH
.... Tec	ELGSGLFGVV	RLGKWRAQYK	VAIKAIREGA	MCEEDFIEEA	KVMMKLTH
DSrc28C	ELGSGQFGVV	RRGKWRSID	TAVKMMKEGT	MSEDDFIEEA	KVMTKLQH

.... Bmx	-SQLLEMCYD	VCEGMAFLES	HQFIHRDLAA	RNCLVDRDLC	VKVSDFGM
.... Btk	-QQLLEMCKD	VCEAMEYLES	KQFLHRDLAA	RNCLVNDQGV	VKVSDFGM
.... Emt	-ETLLGMCLD	VCEGMAYLEE	ACVIHRDLAA	RNCLVGENQV	IKVSDFGM
.... Tec	-DMLLSMCQD	VCEGMEYLER	NSFIHRDLAA	RNCLVNEAGV	VKVSDFGM
DSrc28C	MGLLLDMCIQ	VSKGMTYLER	HNYIHRDLAA	RNCLVGSENV	VKVADFGL

.... Bmx	ILMWEVFSLG	KQPYDLYDNS	QVVLKVSQGH	RLYRPHLASD	TIYQIMYS
.... Btk	VLMWEIYSLG	KMPYERFTNS	ETAEHIAQGL	RLYRPHLASE	KVYTIMYS
.... Emt	VLMWEVFSLG	KIPYENRSNS	EVVEDISTGF	RLYKPRLAST	HVYQIMNH
.... Tec	VLMWEIFTEG	RMPFEKNTNY	EVVTMVTRGH	RLHRPKLATK	YLYEVMLR
DSrc28C	VLMWEIFTCG	KMPYGRCLKNT	EVVERVQRGI	ILEKPKSCAK	EIYDVMKL

TK

SA	GLISRLKYPV	SQQ-NKNAPS	TAGLGYGSWE	IDPKDLTFLK	406
GG	GLVTRRLRYPV	CFG-RQKAPV	TAGLRYGKWV	IDPSELTFVQ	367
AA	GLVTRRLRYPV	STK-GKNAPT	TAGFSYDKWE	INPSELTFMR	270
SG	GL---ACRLK	SSPCDRPVPP	TAGLSHDKWE	IHPIQLMLME	332
..	.....pv	.....	.....	.....	...

PK	LVKFYGVCSK	EYPIYIVTEY	ISNGCLLNLYL	RSHGKGLEP-	510
EK	LVQLYGVCTK	QRPIFIITEY	MANGCLLNLYL	REMRHRFQT-	495
PK	LVQLYGVCTK	QAPICLVFEF	MEHGCLSDYL	RTQRGLFAA-	456
PK	LVQLYGVCTQ	QKPIYIVTEF	MERGCLLNFL	RQRQGHFSR-	359
PN	LVQLYGVCTK	HRPIYIVTEY	MKHGSLNLYL	RRHEKTLIGN	422

Autophosphorylation

TR	YVLDDQYVSS	VGTKFFVKWS	APEVFHYFKY	SSKSDVWAFG	599
SR	YVLDDQYVSS	VGSKFPVRWS	PPEVLMYSKF	SSKSDIWAFFG	584
TR	FVLDDQYVSS	TGTFKFPVKWA	SPEVFSFSRY	SSKSDVWSFG	545
AR	YVLDDQYVSS	SGAKFPVKWC	PPEVFNYSRF	SSKSDVWSFG	448
AR	YVLDDQYVSS	GGTKFPIKWA	PPEVLNYTRF	SSKSDVWAYG	514

CW	HELPEKRPTF	QQLSSIEPL	REKDKH*	.....675
CW	HEKADERPTF	KILLSNILDV	MDEES*	.....659
CW	KERPEDRPAF	SRLLRQLAEI	AESGL*	.....620
CW	QERPEGRPSF	EDLLRTIDEL	VECEETFGR*	...527
CW	SHGPEERPAF	RVLMDQLALV	AQTLTD*	.....590

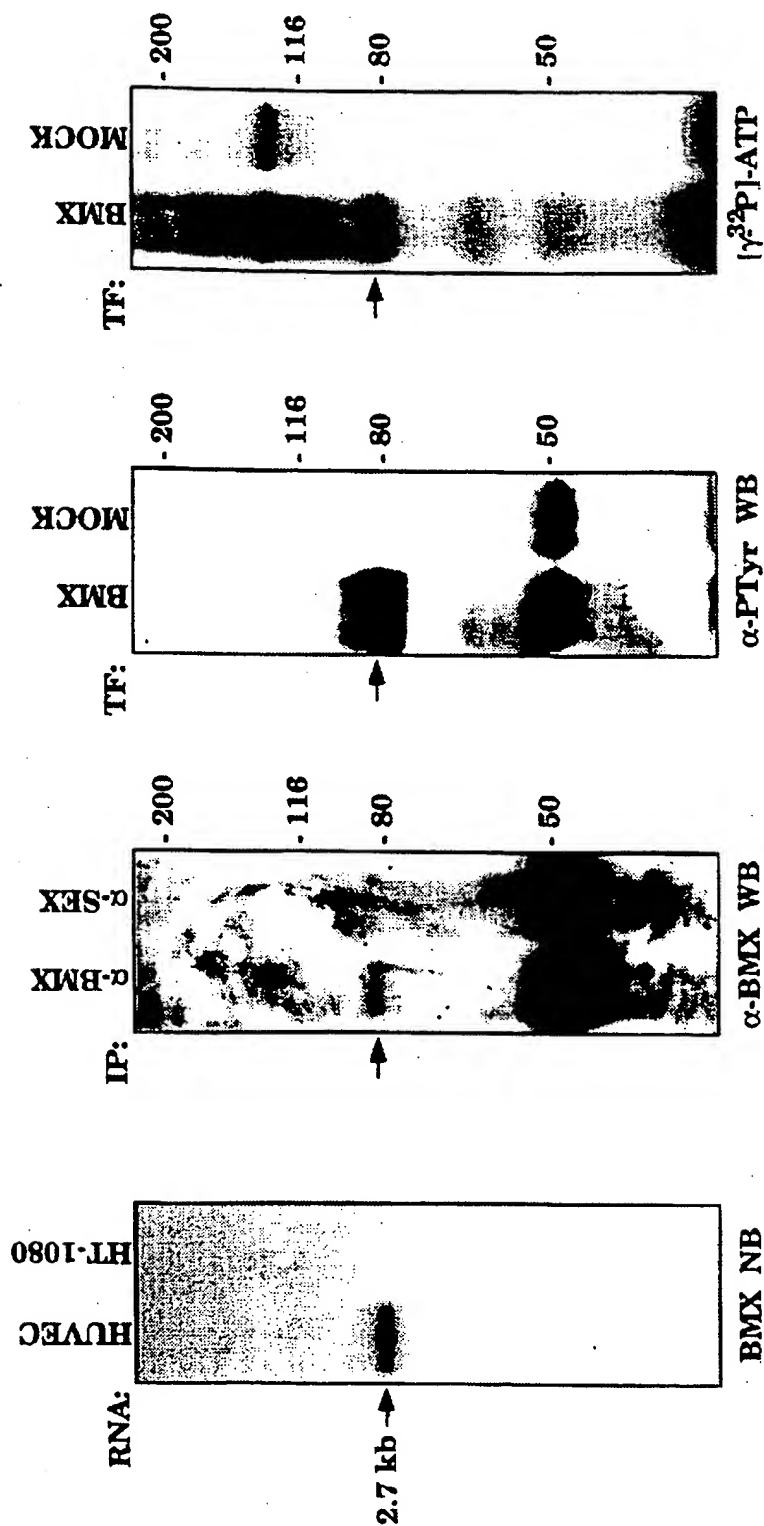


Figure 2D

Figure 2C

Figure 2B

Figure 2A

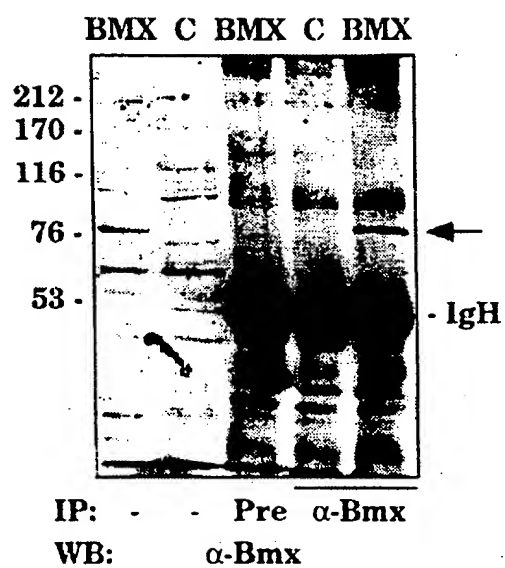


Figure 3

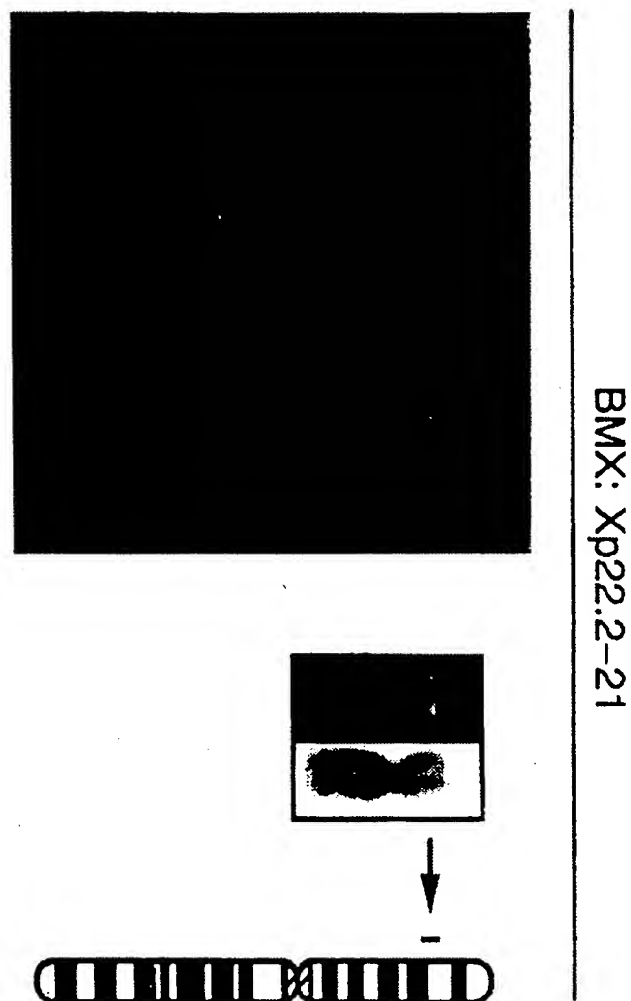


Figure 4

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/FI 95/00555

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/54 C12Q1/68 C07K16/40 C12N9/12

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 86, 1989 WASHINGTON US, pages 1603-1607, A. WILKS 'Two putative protein-tyrosine kinases identified by application of the polymerase chain reaction' see the whole document ---	1-4
A	ONCOGENE (1994), 9(4), 1155-61 CODEN: ONCNES;ISSN: 0950-9232, April 1994 SAKANO, SEIJI ET AL 'Molecular cloning of a novel non - receptor tyrosine kinase, HYL ( hematopoietic consensus tyrosine-lacking kinase)' see the whole document --- -/--	1-5



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents:

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- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- \*&\* document member of the same patent family

Date of the actual completion of the international search

26 February 1996

Date of mailing of the international search report

14.03.96

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# INTERNATIONAL SEARCH REPORT

In International Application No  
PCT/FI 95/00555

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>NATURE, vol. 361, 21 January 1993 pages 226-233, D. VETRIE ET AL 'The gene involved in X-linked agammaglobulinaemia is a member of the src family of protein-tyrosine kinases' cited in the application see the whole document ---</p>	1-5
P,X	<p>ONCOGENE (1994), 9(12), 3683-8 CODEN: ONCNES;ISSN: 0950-9232, December 1994 TAMAGNONE, LUCA ET AL 'BMX, a novel nonreceptor tyrosine kinase gene of the BTK ITK TEC TXK family located in chromosome Xp22.2' see the whole document -----</p>	1-6